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Original Research Article

Antidiabetic and Anti-neuropathic Activities of Hydroalcoholic Polyherbal Extract in Wistar Rats

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ABSTRACT

Diabetes is a chronic condition characterized by high glucose levels and abnormal protein and fat metabolism. Several medicinal plants have been used in the management of diabetes. The present study was conducted to assess the antihyperglycemic and anti-neuropathic activities of a hydroalcoholic extract of a polyherbal mixture (PHM) in diabetic-induced Wistar rats. Diabetes was induced in Wistar ratswith nicotinamide-streptozotocin. A hydroalcoholic extract of a PHM containing *Ocimumtenuiflorum, syzygiumcumini,* and *Aegelmarmelos* was prepared and administered to the diabetic rats. To test for anti-diabetic activity, normal doses of metformin (200 mg/kg), PHM-I (200 mg/kg), PHM-II (400 mg/kg), and PHM-III (800 mg/kg) were administered.To evaluate the neuropathic action of the hydroalcoholicPHM, Pregabalin as a standard dose (50 mg/kg), PHM-I (200 mg/kg), and PHM-II (800 mg/kg) were administered. After giving the medication to the animals for 21 days, neuropathic activity was measured by the animals' pain tolerance and pain frequency using Eddy's hot plate, hot water immersion, and cold allodynia methods. The antihyperglycemic and analgesic effects of PHM were validated by the histopathological analysis. The PHM-III(800 mg/kg) dosage for the treatment of diabetes was more effective. When compared to PHM-II (800 mg/kg), PHM-I (200 mg/kg) showed more analgesic activity in rats. When compared to disease control, PHM-II (800 mg/kg) was the statistically significant ($p<0.001$) dosebetween the two. Therefore, the PHM possesses antidiabetic and anti-neuropathic activitiesand can be a therapeutic potential for managing diabetes.

***Keywords*:** Cold allodynia method, Diabetes,Diabetic neuropathy, Eddy's hot plate method, Polyherbal mixtures, Tail flick method.

Introduction

Diabetes is a long-term disorder with elevated glucose levels and abnormal protein and fat metabolism.[1]Blood sugar levels increased as a result of the pancreas either not producing enough insulin or the cells being unable to adequately absorb the insulin that was being produced.[1]Insulin increases the uptake of glucose by adipose and skeletal muscle cells by directing the glucose transporter GLUT4 to the cell surface and stimulating the liver to store additional glucose as glycogen. This helps get blood glucose levels back to normal. When blood glucose levels are low, the pancreatic cells become activated and release glucagon. To achieve stability, glucagon signals the liver to release glucose into the blood from glycogen reserves.[2]Diabetes mellitus has been classified into two categories: Type I, which is insulin-dependent, and Type II, which is non-insulin-dependent. Type I diabetes is an autoimmune disease characterized by a localized inflammatory response in and around the islets, which is followed by the selective death of insulin-secreting cells.Type II diabetes is distinguished from type I diabetes by peripheral insulin malfunction and resistance.[3]

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There are three distinct types of diabetes. A person with type 1 diabetes, also known as insulin-dependent diabetes mellitus, must consistently inject insulin or use an insulin pump since their body is unable to produce insulin.[4]Another name for this type of diabetes is juvenile diabetes. Type 2 diabetes mellitus, commonly known as non-insulin-dependent diabetes, is a condition caused by insulin resistance, a condition in which cells inappropriately use insulin with or without an absolute insulin deficiency.[5] Some pregnant women develop a physiological disorder as a result of a genetic predisposition. After birth, this kind of diabetes is not always obvious. Some complications ofdiabetesincludediabeticwoundhealing,[5]diabeticnephropathy,[6]diabetic retinopathy,[7]and diabeticneuropathy.[8-10]

In Ayurveda, individual herbs are not sufficient to get the therapeutic effects in the desired way. Toxicity is reduced and therapeutic effect is produced in a better way when the composition with multiple herbs is formulated in a particular ratio.[11]*Ocimumtenurifloru* has the chemical components oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, and -caryophyllene.[12] It has also been proven to have anti-diabetic properties.[13]*Syzygiumcumini* contains isoquercetin, kaemferol, and ellagic acid,[26]glucoside, and anthocyanins, among other chemical components.[14] It was reported that the seeds contain the alkaloid jamboline and the glycoside jambolin or antimellin, which inhibits the diastatic conversion of starch into sugar.[14] Mycaminose, a substance with anti-diabetic properties, is obtained from the seeds of *Syzygiumcumini* plant.[15]

The present study was aimed at investigating the antidiabetic and anti-neuropathic activities of hydroalcoholic polyherbal extract in Wistar rats.

Materials and Methods

Sources of plant materials
The plant materials were obtained from local areas inVisakhapatnam, Andhra Pradesh, India.Early leaf twigs of *Ocimumtenuiflorum*(tulasi), no.25503AUV3,*Syzygiumcumini*(neredu),no.25503AUV3 and *Aegle marmelos*(bilwapatravoucher no.25503AUV1) were collected in February 2023. Dr.S.B.Padal, a plant taxonomist at Andhra University,Visakhapatnam, Andhra Pradesh,India identified and authenticated the plant material. Streptozotocin and nicotinamide were procured from Sainadh Chemicals, Visakhapatnam, Andhra Pradesh, India.

Source of animals
Healthy Wistar rats of the same age, weighing between 150 and 200g were purchased from Sainadh Agency, a reputable breeder in Hyderabad, India.The animals were kept in polypropylene cages in the animal house facility of the institute and were given a normal pellet meal while having access to sufficient food and water (*ad libitum*) and a 12-hour light/dark cycle. The temperature of the cage was maintained at 22°C.

Ethical approval
Ethical approval for this study was obtained from the Vignan Institute of Pharmaceutical Technology (IAEC/VIPT/2022/04). The animals were cared for according to the recommendations for the use and care of laboratory animals (1275/ac/09/CPCSEA) provided by the Committee for Control and Supervision of Experimental Animals (CPCSEA).

Preparation of the hydroalcoholic polyherbal extract
The plant material was dried and powdered. The powder was kept for cold maceration using 70% ethanol (200 mL) and water (60 mL) for 48 hours, vacuum-filtered, and then the obtained semi-solid samples were collected and stored after the distillation process had been completed.[3]

Preparation of test solutions
In each trial, test medications and metformin were weighed based on the animal weights and dissolved in sterile water. Freshly prepared tests and standard solutions were prepared and administered to the rats.[5]

Determination of acute oral toxicity
The acute oral toxicity of a polyherbal formulation was examined using male Wistar rats (150–200g) maintained in normal conditions according to Guideline423 of the Organization for Economic Cooperation and Development (OECD). Animals were maintained for a 12-hour fast before the experiment, receiving only water as needed. Each animal received the test substance via an oral feeding tube, and they were each individually monitored for any indications of toxicity during intervals of 2 hours for 24 hours. Even though the dose was safe up to 2,000 mg/kg, the study only employed a tenth of that.

Induction of diabetes
Animals that were fasted for the entire night (no food, but free access to water) were then given an intraperitoneal injection of nicotinamide 30 minutes before receiving a single intraperitoneal injection of freshly prepared streptozotocin (STZ), which was then dissolved in citrate buffer (55 mg/kg body weight).[26]After 72 hours of streptozotocin administration, increased glucose levels in plasma were used to confirm hyperglycemia.Mice with high blood glucose (200 mg/dL) conformations were used in the study.[16]

Experimental groupings of animals for anti-diabetic activity
Six groups (6 diabetic animals in each) were formed from the diabetic animals at random to investigate the anti-diabetic activity of the hydroalcoholic polyherbal extract.[17] All groups except the normal control group received STZ-nicotinamide.Group I (normal control) received saline treatment; Group II (disease control) was administered STZ(45 mg/kg) and nicotinamide(100mg/kg); Group III received STZ-nicotinamide(i.p.) and PHM-I (200 mg/kg [peroral] body weight; Group IV (STZ-nicotinamide(i.p.) and PHM-II (400 mg/kg [peroral] body weight); Group V got STZ-nicotinamide(i.p.) and PHM-III (800 mg/kg [peroral] body weight); and Group VI (standard group) was administered STZ-nicotinamide(i.p.) and metformin(200mg/kg[p.o.] body weight)[18]

Experimental groupings of animals for neuropathic activity
To investigate the anti-neuropathic activityof the hydroalcoholic polyherbal extract, six groups (six diabetic animals each) were created from the diabetic animals at random. All groups except the normal control group received STZ-nicotinamide.Group I (normal control) received a saline treatment; Group II (disease control) received STZ(45mg/kg) and nicotinamide(100mg/kg; i.p.); Group III was administered STZ-nicotinamide(i.p.) and PHM-I (200mg/kg [peroral] body weight); Group IV was given STZ-nicotinamide(i.p.) and PHM-II (800 mg/kg [peroral] body weight); GroupV (standard group)received STZ-nicotinamide(i.p.) and Pregabalin (50 mg/kg body weight [p.o.] and metformin(200mg/kg [peroral] body weight.The animals underwent Eddy's hot plate and tail flick procedures. A sciatic nerve ligation was performed one week before the last day of administration of the test drug formulations.[18] Body weight was monitored throughout the trial.

Evaluation of behavioral reactions using Eddy's hot plate method
Wistar rats that were between 150 and 200 g in weight were used for this procedure. A hot plate was maintained at a temperature of 55 to 56^{o}C. The rats were initially kept in the cabin, where they were observed for behaviors, such as jumping, paw withdrawal, and paw licking. The time between when the rats were placed on the hot plate and when they responded was measured using a timerfor 9 hours at intervals of 1 hour. The results were recorded.[19]
Evaluation of behavioral reactions using the cold allodynia method
The acetone drop test was used to examine behavioral reactions to harmless cold stimuli. The unilateral mid-plantar hind paw received a 50 µl droplet of acetone through a thin polyethylene tube attached to a syringe. Following ten applications of acetone spaced roughly three minutes apart from one another, the number of foot withdrawals was counted. A rapid foot withdrawal response following the application of acetone to the paw's plantar area was observed as proof of cold allodynia at regular intervals of 1 hour for a total of nine hours.[20]

Evaluation of behavioral reactions using the tail immersion method
The tail immersion strategy was used to examine the main mechanism of the analgesic effect. The animals' tail tips were placed in hot water to create thermal stimulation. To measure the baseline reaction time, the rats' tail ends (the last 1-2 cm) were immersed in hot water heated to 55±1°C at regular intervals of 1 hour for a total of 9 hours. The withdrawal of the tail was noted.[21]

Histopathological analysis
The tissue samples were prepared for histopathological analysis by fixation, embedding, sectioning, and staining. Then, the prepared tissues were viewed under a light microscope, and the results were interpreted by a pathologistin Visakhapatnam, India.

Statistical analysis
Graph Pad Prism software (version 5.01) was used to perform the statistical analysis. The mean comparison was done using the two-way analysis of variance (ANOVA).The values are expressed as mean $\pm$ standard error of the mean (SEM).

Results and Discussion

Managing fasting blood sugar levels involves a combination of lifestyle changes and, in some cases, medication. Lifestyle factors that influence fasting blood sugar levels include diet, exercise, stress management, and sleep.[2] Medications such as insulin and oral antidiabetic drugs may be prescribed to help regulate blood sugar levels.There are reports of some medicinal plants that have been used for the management of diabetes.[9,11,12] Using polyherbal mixtures in the management of diabetes can offer several potential advantages,

although it is important to note that scientific evidence supporting the effectiveness of specific PHM formulations can vary. Synergistic effects, improved blood sugar control, and antioxidant and anti-inflammatory effects are some of the advantages put forward to support the use of PHM in the management of diabetics.One strategy for producing an efficient phytochemical combination is to mix various hypoglycemic and hypolipidemic herbs to produce a more potent anti-diabetic medication. The antidiabetic effect is often achieved when the plant extract is administered at high doses, however, this may be accompanied by unfavorable side effects in the body.[22] In the present study,hydroalcoholicPHM was prepared using *Ocimumtenuiflorum*(tulasi), *Syzygiumcumini*(neredu), and *Aegle marmelos*(bilwapatra), and its antidiabetic activity was evaluated in Wistar rats.

Monitoring fasting blood sugar levels helps individuals and healthcare providers make informed decisions about diabetes management. Regular monitoring can reveal trends and patterns in blood sugar control, which can guide treatment adjustments, medication changes, dietary modifications, and exercise routines. In the present study, the results obtained for the effect of the hydroalcoholic PHM on FBS levels of Wistar rats on the 7th and 21st days of diabetic induction are presented in Table 1. The FBS levels were comparable in the hydroalcoholic PHM treatment to the standard medication used in the treatment of diabetes for both the 7th and 21st days of diabetic induction. The decrease in blood glucose may be attributed to the scaryophyllene and rosmarinic acidic contents of the hydroalcoholic PHM.

Neuropathic protection in diabetes refers to strategies and interventions aimed at preventing, managing, and reducing the risk of diabetic neuropathy. Diabetic neuropathy is a common complication of diabetes that affects the nerves, leading to symptoms such as pain, numbness, tingling, and loss of sensation, usually starting in the feet and later progressing to other areas of the body. Neuropathy occurs due to prolonged high blood sugar levels that damage the blood vessels that supply the nerves and the nerves themselves.

The evaluation of the neuropathic protective effects of the hydroalcoholic PHM on diabetic-induced Wistar rats was investigated using various methods. When Eddy's hot plate method was employed to investigate the neuropathic protective effects of the hydroalcoholic PHM (Table 2 and Figure 1), it was observed that pain latencies were comparable in all the groups. Analgesic activity in hydroalcoholi cPHM-Iwas statistically significant ($p < 0.01$) when compared to the control group. More so, the analgesic activity in hydroalcoholic PHM-Iwas statistically significant ($p < 0.05$) when compared to the standard group. A similar observation was made when the neuropathic protective effect of the hydro alcoholicPHM was investigated using the hot water immersion method on the 21st day of diabetic induction (Table 3 and Figure 2).

Table 1: Fasting blood sugar levels on the 7th and 21st days of diabetic induction in Wistar rats.

Group	Day of induction	
	7th	21st
NC	86.3 ± 2.5	80.00 ± 1.8
DC	216.0 ± 0.8$^{\#}$	362.50 ± 3.3$^{\#}$
Standard (200 mg/kg)	214.33 ± 13*	106.33 ± 3.5***
PHM-I (200 mg/kg)	218.33 ± 0.7*	136.33 ± 4.4*
PHM-II (400 mg/kg)	218 ± 0.5**	130.33 ± 4.5**
PHM-III (800 mg/kg)	216.66 ± 1.4***	122.5 ± 3.0***

Statistical analysis was performed using Bonferroni posthoctest two-way analysis of variance (ANOVA); *:$p<0.05$ when comparing PHM1 with disease control;**:$p<0.01$when comparing PHM2 with disease control; ***:$p<0.001$when comparing PHM-III with disease control;***: $p<0.001$when comparing disease control with normal control; $^{\#}$:NC: Normal control; DC: Diseased control; PHB: Polyherbal mixture treatment.

As observed in the results of the neuropathic protective effect of the hydroalcoholicPHM using the cold allodynia method (Table 4 and Figure 3), it was also observed that pain latencies were comparable in all the groups. Analgesic activity in hydroalcoholic PHM-Iwasstatistically significant($p<0.05$) when compared to the control group and also statistically significant ($p<0.001$)when compared to the disease control group.The results of the histopathological analysis (Figure 4) showan increase in beta cell function in the pancreas of the PHM-III treatedgroupwhen compared to hydroalcoholicPHM-II and PHM-I, and regeneration of beta cells of the islets of Langerhans in the hydroalcoholicPHM-III group when compared to PHM-II and PHM-I.Due to the presence of carvacrol, kaemferol, and myrecetin in PHM, neuropathy has analgesic and anti-inflammatory effects.[23,]

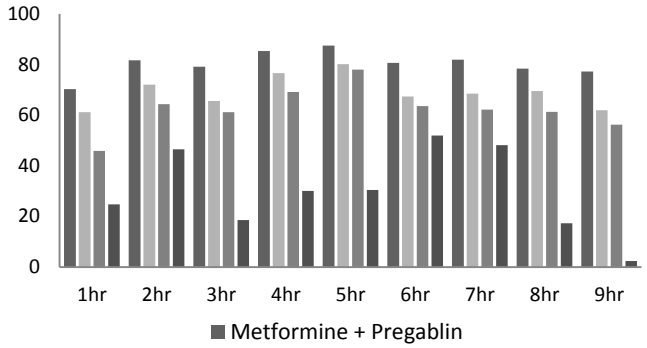

Figure 1: Percentage of neuropathic protection in Wistar rats measured by Eddy's hot plate method on the 21st day of diabetic induction.

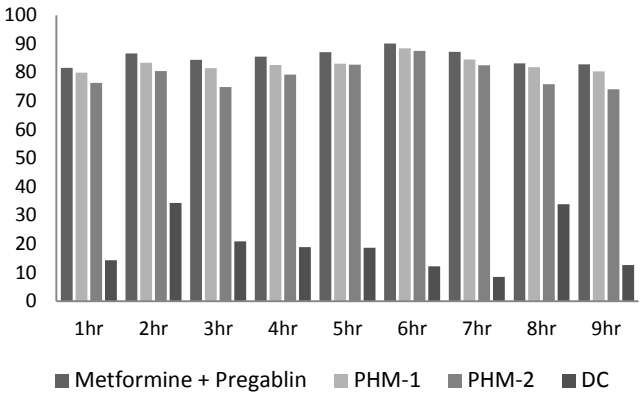

Figure 2: Percentage of neuropathic protection in Wistar rats measured by hot water immersion method on the 21st day of diabetic induction.

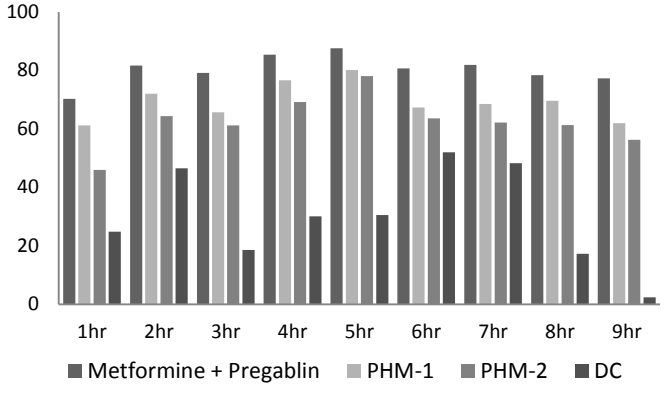

Figure 3: Percentage of neuropathic protection in Wistar rats measured by cold allodynia method on the 21st day of diabetic induction.

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Table 2: Neuropathic activity of experimental animals measured by Eddy's hot plate method on the 21st dayof diabetic induction.

Group	Time (hour)							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
NC	2.49	2.5	2.49	2.51	2.48	2.13	2.12	2.15
DC	3.13 ± 0.04$^{\#}$	4 .56 ± 0.25$^{\#}$	3.45 ± 0.07$^{\#}$	3.67 ± 0.07$^{\#}$	4.34 ± 0.06$^{\#}$	4.68 ± 0.08$^{\#}$	4.58 ± 0.10$^{\#}$	4.25 ± 0.07$^{\#}$
METFORMIN + PREGABLIN (200mg/kg and 50mg/kg)	18.57 ± 0.09***	20.38 ± 0.05***	13.87 ± 0.03***	25.37 ± 0.05***	26.26 ± 0.04***	26.68 ± 0.06***	25.78 ± 0.07***	23.15 ± 0.08***
PHM-I(200mg/kg)	15.89 ± 0.06**	16.47 ± 0.26**	17.48 ± 0.37**	18.59 ± 0.07**	18.89 ± 1.09**	16.47 ± 0.04**	15.58 ± 0.47**	15.89 ± 0.07**
PHM-II(800mg/kg)	12.67 ± 0.89***	14.67 ± 0.47***	14.98 ± 0.59***	15.67 ± 0.76***	16.38 ± 0.26***	17.67 ± 0.04***	18.67 ± 0.48***	19.58 ± 0.69***

NC: Normal control; DC: Diseased control; PHB: Polyherbal mixture treatment; Pain latencies were comparable in all the groups. Analgesic activity in PHM-I is statistically significant when compared to the control group, **: p<0.01; Analgesic activity in PHM-I is statistically significant when compared to standard group,*: p<0.05; NC: Normal control; DC: Diseased control; PHB: Polyherbal mixture treatment.

Table 3: Neuropathic activity of experimental animals measured by hot water immersion method on the 21st day of diabetic induction

Group	Time (hour)								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
NC	2.76	2.45	2.89	3.12	2.89	2.24	2.78	3.45	3.28
DC	3.67 ± 0.02$^{\#}$	4.58 ± 0.56$^{\#}$	3.16 ± 0.86$^{\#}$	4.46 ± 0.19$^{\#}$	4.16 ± 0.75$^{\#}$	4.67 ± 0.45$^{\#}$	5.37 ± 0.76$^{\#}$	4.17 ± 0.78$^{\#}$	3.36 ± 0.65$^{\#}$
METFORMIN + PREGABLIN (200and 50mg/kg)	15.17 ± 0.56	17.57 ± 0.87	18.58 ± 0.67	21.58 ± 0.65	22.48 ± 0.83	22.68 ± 0.06	21.69 ± 0.46	20.48 ± 0.06	9.12 ± 0.01
PHM1(200mg/kg)	13.78 ± 0.56**	14.78 ± 0.56**	16.58 ± 0.12**	18.37 ± 1.8**	18.59 ± 0.29**	19.45 ± 2.2**	17.98 ± 0.56**	18.94 ± 0.53**	18.02 ± 0.34**
PHM2(800mg/kg)	11.67 ± 0.42	12.53 ± 0.41	13.69 ± 0.32	15.05 ± 0.54	16.78 ± 0.02	17.94 ± 0.34	15.93 ± 0.42	14.32 ± 0.42	13.73 ± 0.27

NC: Normal control; DC: Diseased control; PHB: Polyherbal mixture treatment.

Table 4: Neuropathic activity of experimental animals measured by cold allodynia methodon the 21st day of diabetic induction

Group	Time (hour)								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
NC	3.67	*2.87*	3.67	2.78	3.56	3.78	3.36	2.67	2.62
DC	3.52 ± 0.13$^{\#}$	4.37 ± 0.24$^{\#}$	3.67 ± 0.52$^{\#}$	3.43 ± 0.21$^{\#}$	4.38 ± 0.24$^{\#}$	3.37 ± 0.73$^{\#}$	3.67 ± 0.18$^{\#}$	4.04 ± 0.34$^{\#}$	4.57 ± 0.19$^{\#}$
METFORMIN + PREGABLIN(200mg/kg+50mg/kg)	12.36 ± 0.53***	15.67 ±	17.62 ± 0.35***	19.04 ± 1.5***	20.56 ± 1.8***	19.58 ± 0.38***	18.57 ± 0.74***	17.58 ± 0.45***	16.68 ± 0.24***
PHM-I(200mg/kg)	9.45 ± 0.34***	10.28 ± 0.56***	10.67 ± 0.54***	11.89 ± 0.32***	12.93 ± 0.86***	11.59 ± 0.86***	10.69 ± 0.24***	8.78 ± 0.14***	6.89 ± 0.21***
PHM-II(800mg/kg)	6.79 ± 0.64*	8.05 ± 0.46*	9.45 ± 0.86*	10.05 ± 0.78*	11.67 ± 0.10*	10.39 ± 0.86*	8.89 ± 0.68*	7.69 ± 0.76*	5.99 ± 3 ± 0.75*

Pain latencies were comparable in all the groups. Analgesic activity in PHM-Iwas statistically significant when compared to the control group,*: p<0.05; Analgesic activity in PHM-Iwas statistically significant when compared to the disease control group,*: p<0.001; NC: Normal control; DC: Diseased control; PHB: Polyherbal mixture treatment.

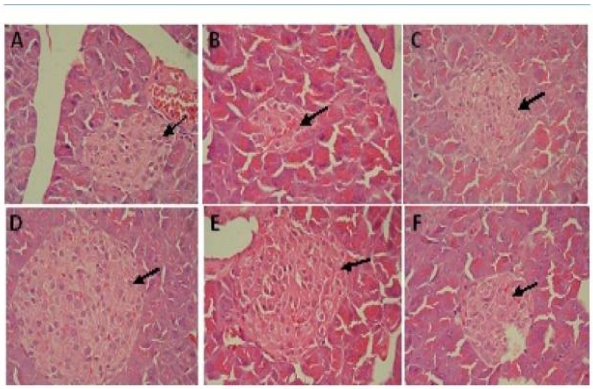

Figure 4: Histopathological analysis of pancreas in nicotinamide-streptozotocin-induced diabetic Wistar rats treated with different doses of the polyherbal mixture.
A: Normal; B: Standard (Metformin);C: PHM-I(200mg/kg); D: PHM-II(400mg/kg); E: PHM (800mg/kg); F: Diseased control.

As observed in the results of the neuropathic protective effect of the hydroalcoholicPHM using the cold allodynia method (Table 4 and Figure 3), it was also observed that pain latencies were comparable in all the groups. Analgesic activity in hydroalcoholicPHM-Iwas statistically significant($p<0.05$) when compared to the control group and also statistically significant ($p<0.001$)when compared to the disease control group.The results of the histopathological analysis (Figure 4) showan increase in beta cell function in the pancreas of the PHM-III treatedgroupwhen compared to hydroalcoholicPHM-II and PHM-I, and regeneration of beta cells of the islets of Langerhans in the hydroalcoholicPHM-III group when compared to PHM-II and PHM-I.Due to the presence of carvacrol, kaemferol, and myrecetin in PHM, neuropathy has analgesic and anti-inflammatory effects.[23,24]

Conclusion

The hydroalcoholicPHM-III was more effective against diabetic Wistar rats compared to PHM-II, and PHM-I1. Therefore, the order of effectiveness is PHM-III>PHM-II>PHM-I. When the analgesic activity of the hydroalcoholicPHMwas examined by hot water immersion, Eddy's hot plate,and cold allodynia techniques, it was discovered topossess neuropathic protective potential in the rats. The main constituent that was responsible for analgesic activity in the hydroalcoholicPHM was carvacrol. The order of analgesic effect was PHM-I>PHM-II>PHM-III.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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